

EXHIBIT ‘F’

Molecular Cloning of a Human Prion Protein cDNA

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ABSTRACT

Creutzfeldt-Jakob disease (CJD) of humans and scrapie of animals are degenerative, transmissible neurologic diseases caused by prions. The only known macromolecules within prions are prion proteins (PrP). The cDNA encoding the hamster prion protein (PrP 27-30) has been cloned and sequenced (Oesch *et al.*, 1985). Using that hamster PrP cDNA, we screened a human retina cDNA library and sequenced the cDNA clone with the longest hybridizing insert. This insert was found to contain a long open reading frame (ORF) encoding the human prion protein. Northern transfer analysis showed that a related poly(A)⁺RNA measuring ~2.5 kb is expressed in a variety of human neuroectodermal cell lines. Human PrP differed from hamster PrP at 27 of 253 amino acids and at 98 of 759 ORF nucleotides. Conservation of PrP amino acid sequence between hamster and human is nearly 90%, reflecting similar structural features and shared antigenicity of the two proteins (Bockman *et al.*, 1985). The human PrP sequence contained a presumptive amino-terminal signal peptide of 22 amino acids, two hydrophobic segments of sufficient length to span membranes, and two possible sites for *N*-glycosylation. The conservation between the hamster and human prion proteins suggests that they may have an important role in cellular metabolism and may explain the similarities between scrapie and CJD.

INTRODUCTION

CREUTZFELDT-JAKOB DISEASE (CJD), Gerstmann-Sträussler syndrome (GSS), and kuru in man and scrapie in animals have been grouped together because they are transmissible diseases and have similar histopathologic and clinical features (Hadlow, 1959; Gajdusek, 1977; Masters *et al.*, 1981). Common histological features are spongiform degeneration of central nervous system (CNS) neurons, intense reactive astrocytic gliosis, and amyloid plaque formation (Beck and Daniel, 1979; Kitamoto *et al.*, 1986). The only cell type which appears to be injured directly in these diseases is the CNS neuron and the only cell type which appears to react to the diseases is the astrocyte. Except for transmissibility, none of the clinical or histological features is characteristic of a conventional infectious pathogen such as a virus. No virus has been demonstrated by electron microscopy and none has been isolated by cell culture procedures. In addition, there is no inflammatory or immune response during the course of these diseases (Zlotnik and Stamp, 1961; Beck and Daniel, 1979; Kasper *et al.*, 1982).

Highly purified preparations of the scrapie agent consist primarily of a 27- to 30-kD sialoglycoprotein, which is inseparable from infectivity (Bolton *et al.*, 1982; Prusiner *et al.*, 1982, 1983; McKinley *et al.*, 1983). In contrast to viruses, no nucleic acid has been identified to date in these purified preparations. To distinguish the infectious agents causing CJD, GSS, kuru, and scrapie from viruses, the term "prion" was introduced (Prusiner, 1982). The protein in purified preparations of prions was designated "PrP 27-30" (McKinley *et al.*, 1983).

Following the isolation of a cDNA encoding PrP 27-30 (Oesch *et al.*, 1985), the origin and site of synthesis of the prion protein was established. PrP 27-30 was found to be encoded by a cellular gene in hamsters. The gene product in normal animals is a 33- to 35-kD protein termed "PrP 33-35^C". This protein is also present in scrapie-infected hamsters (Meyer *et al.*, 1986); however, an abnormal isoform also appears in the CNS during the course of scrapie which has been termed "PrP 33-35^{Sc}" (Oesch *et al.*, 1985; Barry *et al.*, 1986; Meyer *et al.*, 1986). PrP 33-35^{Sc} differs from the normal cellular isoform in three ways: First, the concentration of PrP 33-35^C does not change with age in

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normal or scrapie-infected animals while the concentration of PrP 33-35^{Sc} increases 10-20 times during scrapie infection. Second, the cellular isoform is rapidly digested by proteinase K, whereas the PrP 33-35^{Sc} isoform is only partially digested yielding PrP 27-30. PrP 27-30 was the only form of the prion protein found in highly purified preparations of the scrapie agent because of a proteinase K digestion step in the purification procedure. Third, although both PrP 33-35^C and PrP 33-35^{Sc} are membrane proteins, upon detergent extraction PrP 33-35^C is solubilized while PrP 33-35^{Sc} polymerizes into amyloid rods (Meyer *et al.*, 1986).

In situ hybridization with the hamster PrP cDNA encoding PrP 27-30 indicates that the prion protein isoforms are synthesized almost exclusively in CNS neurons since there are 10 to 50 PrP mRNA copies per neuron and less than three copies per cell in glial cells and nonneural cells (Kretzschmar *et al.*, 1986). Recent immunohistochemical studies with a monoclonal antibody specific for the prion protein (Barry and Prusiner, 1986) indicate that both the normal cellular and scrapie isoforms are located in CNS neurons and their processes (S.J. DeArmond, D. DeMott, R.A. Barry, H.A. Kretzschmar, and S.E. Prusiner, in preparation). Late in the course of the disease, the scrapie isoform polymerizes into filaments and accumulates in the CNS extracellular space coalescing into amyloid plaques (Bendheim *et al.*, 1984; DeArmond *et al.*, 1985; Kitamoto *et al.*, 1986).

The number of PrP mRNA copies are the same in each nerve cell population throughout the course of scrapie and are not different than in normal animals (Kretzschmar *et al.*, 1986). This is in agreement with Northern blot measurements of PrP mRNA in hamsters (Oesch *et al.*, 1985) and in mice (Chesebro *et al.*, 1985). These findings raised the possibility that PrP 33-35^{Sc} is generated by post-translational modification.

Multiple experimental observations suggest that the etiologic agents causing CJD, GSS, and kuru are closely related to the prions causing scrapie in animals. First, antibodies raised against hamster PrP 27-30 react with proteinase K-resistant proteins purified from patients with CJD (Bockman *et al.*, 1985). Second, amyloid plaques in CJD and GSS specifically bind antibodies raised against hamster PrP 27-30 (Kitamoto *et al.*, 1986). Third, the hamster PrP cDNA identifies a related single-copy sequence in human genomic DNA (Oesch *et al.*, 1985).

In order to study the pathogenesis of human prion diseases further, it was necessary to establish the molecular structure of the human prion protein. We isolated PrP-related clones from a human retina cDNA library (Nathans *et al.*, 1986). A 2.9-kb clone (HuPrPcDNA-1) was completely sequenced and a long open reading frame (ORF) encoding the prion protein was identified. Another 2.4-kb clone (HuPrPcDNA-2) was partially sequenced, and a cloning artefact in the untranslated 5' region of the longer clone was defined. The human prion protein consists of 231 amino acids preceded by a presumptive signal peptide of 22 amino acids. The human prion protein exhibits 87% homology with the hamster protein.

MATERIALS AND METHODS

A human retina cDNA library constructed in λ gt10 (Nathans and Hogness, 1983; Huynh *et al.*, 1985) from a total of 25 different individuals was obtained from J. Nathans at Stanford University. The library was screened using a hamster PrP cDNA probe under conditions of reduced stringency (Shank *et al.*, 1978; Oesch *et al.*, 1985). Hybridization was performed at 42°C in 3× SSC, 30% formamide, 0.05 M HEPES pH 7.4, 0.2 mg/ml salmon sperm DNA, 0.15 mg/ml yeast RNA, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidone, and 0.02% Ficoll. Filters were washed in 0.1× SSC, 0.1% NaDodSO₄, at 50°C and autoradiographed at -70°C using Dupont Cronex intensifying screens and Kodak XAR-5 film.

Phage inserts were excised with *Eco* RI. The longest insert (~2.9 kb, HuPrPcDNA-1) was propagated in pUC8 (Vieira and Messing, 1982), digested with *Eco* RI and purified on a 1% agarose gel. It was then shotgun-cloned into M13mp18 (Norlander *et al.*, 1983) using *Rsa* I, *Alu* I, *Hae* III, *Fnu* DII, *Pst* I, and *Sma* I digests. Large subclones were cut back with T4 polymerase and religated in M13 as described (Dale *et al.*, 1985). HuPrPcDNA-1 was sequenced mostly on both strands (Fig. 1) using the dideoxy protocol (Sanger *et al.*, 1977; Biggin *et al.*, 1983). A 17-mer oligonucleotide was synthesized on an Applied Biosystems Model 380B DNA Synthesizer and was used as a primer to sequence a gap in the 3' noncoding region (Fig. 1). A smaller insert (~2.4 kb, HuPrPcDNA-2) also was propagated in pUC8. The 5' and 3' ends, as well as the 3' end of the ORF, of this clone were "supercoil" sequenced (Chen and Seeburg, 1985). All sequences were read by two of the authors.

Poly(A)⁺-enriched RNAs from human cell lines were a gift from Manfred Schwab (G.D. Hooper Foundation, University of California, San Francisco; Schwab *et al.*, 1984) and poly(A)⁺-enriched RNA from hamster glioma cell line HJC was a gift from D. Butler (University of California, San Francisco). Poly(A)⁺ RNA was electrophoresed on 2.2 M formaldehyde, 1% agarose gels, and transferred to nitrocellulose (Thomas, 1980), which then was baked and hybridized with ³²P-labeled hamster ORF (Westaway and Prusiner, 1986) or full-length human cDNA probes. Approximately 10⁶ cpm of nick-translated probes (Rigby *et al.*, 1977) were added per milliliter of hybridization fluid. Hybridization conditions with the hamster PrP cDNA probe were as described above for screening the cDNA library in which 50% formamide was used. With the human PrP cDNA probe, 40% formamide was used.

RESULTS

Previous hybridization (Oesch *et al.*, 1985) and immunohistochemical studies (Bockman *et al.*, 1985) have implied that the human genome contains a PrP gene. As a prelude to cDNA cloning, we sought a PrP mRNA in human poly(A)⁺ RNA isolated from a number of neuroectodermal cell lines to determine the size of the PrP mRNA. mRNA was

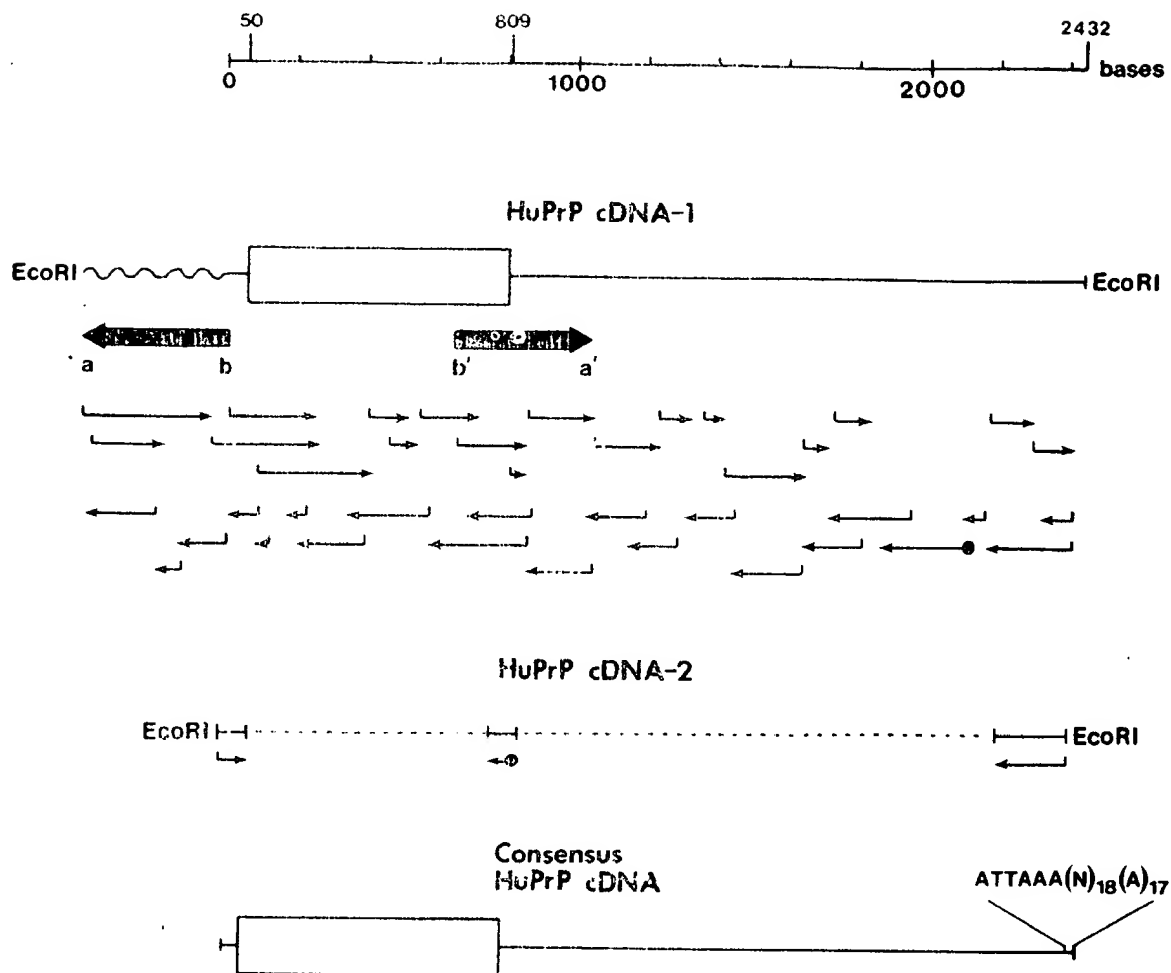


FIG. 1. Sequencing strategy. ORFs are shown as boxes. Bold arrows and wavy line in HuPrP cDNA-1 represent stretches of inverted repeat-sequences. Thin arrows denote sequenced M13 subclones. Dark circles symbolize specially synthesized sequencing primers. The 5' and 3' ends as well as the 3' end of the ORF of the HuPrP cDNA-2 were "supercoil" sequenced. A consensus sequence was established by exclusion of incongruent sequences at the 5' ends of HuPrPcDNA-1 and HuPrPcDNA-2 (see also Fig. 3).

electrophoresed on a 1% agarose gel and transferred to nitrocellulose (Thomas, 1980). A hamster PrP cDNA subclone corresponding to the ORF (Westaway and Prusiner, 1986) was radiolabeled and hybridized. A single RNA species of ~2.5 kb was observed in all the human poly(A)⁺RNA samples on a Northern transfer (Fig. 2A, lanes 1-5). A hamster glioma cell line poly(A)⁺RNA was electrophoresed as a control and exhibited a single species of 2.2 kb (Fig. 2A, lane 6), a size which is in agreement with previous Northern transfers (Oesch *et al.*, 1985).

A human retina cDNA library was screened using a hamster PrP cDNA probe (Oesch *et al.*, 1985), and nine positive clones were identified and selected. Their inserts were excised with *Eco*RI and analyzed by agarose gel electrophoresis. The PrP-related inserts ranged in size from 1.4 to 2.9 kb. The longest insert, measuring 2.9 kb (HuPrPcDNA-1), was subcloned into pUC8 (Vieira and

Messing, 1982), purified, and radiolabeled. It was hybridized to aliquots of the same human poly(A)⁺RNA samples used above. This probe detected transcripts of the same electrophoretic mobility as those detected by the hamster PrP probe (Fig. 2B, lanes 1-5). We attribute the reduced hybridization of the human PrP cDNA with the hamster poly(A)⁺RNA control (lane 6) to two factors. First, the higher stringency conditions used in this analysis (40% vs. 30% formamide); second, the full-length human cDNA used as a probe includes 1624 cDNA nucleotides of the 3' untranslated region which is 436 bases longer than the hamster and has only 22% homology with the hamster cDNA based on computer analysis of optimal alignment of stretches of 8 identical nucleotides in both sequences. In contrast, the 720-bp hamster PrP probe fragment is derived mainly from the ORF, which is highly conserved.

The congruent pattern of hybridization to human poly-

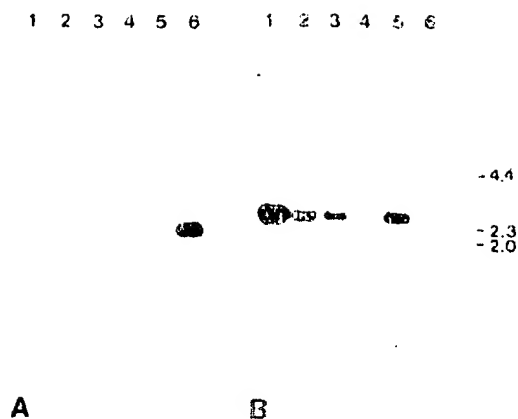


FIG. 2. RNA transfer analysis. Lane 1: Human melanoma RPMI-7951; lane 2: human melanoma HT-144; lane 3: human neuroblastoma Kelly; lane 4: human neuroblastoma NMB; lane 5: human retinoblastoma Y79; lane 6: hamster glioma HJC. Five micrograms of polyadenylated RNA were analyzed in each lane. A radiolabeled *Hind* III digest of phage λ was used as size-marker. **A.** Hybridization with a hamster PrP cDNA subclone corresponding to the ORF (Westaway and Prusiner, 1986) (30% formamide). **B.** Hybridization using the entire human PrP cDNA clone (40% formamide).

(A) RNA obtained with both the hamster and human probes establishes the authenticity of the human 2.9-kb insert (HuPrPcDNA-1). Furthermore, we conclude that PrP poly(A)⁺ RNA is expressed in a variety of human neuroectodermal cell lines.

The 2.9-kb insert (HuPrPcDNA-1) was then shotgun-cloned into M13mp18 and sequenced using the dideoxy protocol (Sanger *et al.*, 1977; Biggin *et al.*, 1984) (Figs. 1 and 3). Computer analysis (Conrad and Mount, 1982) revealed that the first 407 nucleotides of this clone contained the exact complement of a stretch of 400 nucleotides further downstream (nucleotides 651–1050 in Fig. 3). Similar rearrangements in cDNAs have been described previously and models have been proposed to explain their appearance (Fields and Winter, 1981; Vellekaert *et al.*, 1981). Specifically, this sequence rearrangement (Fig. 4) could have arisen during first-strand synthesis through a folding back of the first cDNA strand on itself and use of further downstream sequences as a template for 5' extension. After unfolding of this loop (via denaturation) and formation of a hairpin-loop at the new 5' end, second-strand synthesis would then have proceeded in the usual way. To delineate the 5' untranslated region of the human PrP mRNA, the 5' end of a 2.4-kb clone (HuPrPcDNA-2) was sequenced. Close to the 5' end, this clone contained a short inverted repeat corresponding to nucleotides 18–26 in Fig. 3. This repeat might represent an artefact similar to HuPrPcDNA-1. A consensus cDNA sequence was established by excluding incongruent sequences at the 5' ends of the HuPrPcDNA-1 and HuPrPcDNA-2 (Figs. 1, 3, and 4). The total length of

the consensus cDNA sequence is 2432 nucleotides, in good agreement with the mRNA size estimated from Northern blot analysis (2.5 kb).

Close to the 5' end of the consensus sequence there are two ATG codons beginning at nucleotides 50 and 71. The nucleotide sequence surrounding the first ATG codon (ATTATGG) is consistent with the consensus sequence (ANNATGG) for eukaryotic initiation sites (Kozak, 1983). The sequence surrounding the ATG codon beginning at nucleotide 71 (TGGATGC) deviates from this consensus pattern. Thus, we conclude that the most likely site for initiation of translation is the ATG codon beginning at nucleotide 50. Following the putative initiation codon is a long ORF of 759 bases.

The 3' noncoding region consists of 1624 nucleotides which includes numerous in-frame termination signals and 17 A residues at the 3' end. Two possible polyadenylation signals are found at positions 2356 (TATAAA) and 2392 (ATTAAA). Both are variants of the consensus sequence AATAAA (Proudfoot and Brownlee, 1976) which have been described previously for the hepatitis-B surface antigen gene (TATAAA; Simonsen and Levinson, 1983) and the chick α -actin gene (ATTAAA; Fornwald *et al.*, 1982), respectively. The ATTAAA at position 2392 precedes the polyadenylation tract by 18 nucleotides and most likely represents the polyadenylation signal.

The human PrP, predicted from this cDNA sequence, consists of 253 amino acids with a molecular weight of 27,665.67 prior to post-translational modification. The amino terminus displays a segment of 22 residues that are typical of signal peptides (von Heijne, 1985). These include a hydrophobic core (MLVLFV) and a small uncharged residue (C) at the putative signal sequence cleavage site; we predict that the mature protein commences at the lysine residue at nucleotide 116, and prior to post-translational modification has a molecular weight of 25,239 daltons.

DISCUSSION

Two cDNA clones encoding the human prion protein were isolated from a human retina cDNA library. A consensus nucleotide sequence was derived from these two clones. A comparison with the hamster cDNA (Oesch *et al.*, 1985) shows 87.1% homology in the ORF. The 3' noncoding region of the human cDNA is 436 nucleotides longer than the corresponding hamster sequence, and the degree of homology within this region is considerably lower (22%) than in the ORF (Fig. 5).

The predicted amino acid sequence of the human PrP protein was aligned with the hamster PrP sequence in Fig. 6 (Oesch *et al.*, 1985; K. Basler, B. Oesch, M. Scott, D. Westaway, M. Wälchli, D.F. Groth, M.P. McKinley, S.B. Prusiner, and C. Weissmann, submitted). The protein sequences differ in length by one amino acid (253 in human vs. 252 in hamsters). In addition, 26 amino acid residues differ between the hamster and the human sequences. This sequence divergence (10.7%) is paralleled by 98 out of 759 (12.9%) variation in the nucleotide sequence. Fourteen of these are mismatches in the first position, 14 are mis-

1 30 60 90
 C GGC GCC GCG AGC TTC TCC TCT GCT CAC GAC CGA GCG AGA GCA TTC ATT ATG GCG AAC GTT GCG TGC TGG ATG CTG GTT CTC TTT CTG GCC ACA TGG AGT GAC
 MET Ala Asn Leu Gly Cys Trp MET Leu Val Leu Phe Val Ala Thr Trp Ser Asp
 120 150 180
 CTG GCG CTC TGC AAG AAG GCG GCG AAG CCT GGA GGA TGG AAC ACT GGG GCG ACC GCA TAC CCG GCG CAG GGC AGC CCG GGA GGC AAC CCG TAC CCA CCT CAG GCG
 Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg Tyr Pro Pro Gln Gly
 210 240 270 300
 GGT GGT GCG TGG GCG CAG CCG CAT GCT GGT GCG TGG GCG CAG CCG CAT GGT GGT GCG TGG GCG CAG CCG CAT GGT GGT GCG TGG GCG CAG CCG CAT GGT GGT GCG
 Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His Gly Gly
 330 360 390 420
 TGG GGT CAA GGA GGT GCG ACC CAG AGT CAG TGG AAC AAG CCG AGT AAG GCA AAA ACC AAC ATG AAG CAC ATG GCT GGT GGT GCG CCA GCA CCG GGA GCA GTG GTG GCG
 Trp Gly Gln Gly Gly Thr His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn MET Lys His MET Ala Gly Ala Ala Ala Gly Ala Val Val Gly
 450 480 510
 GCG CTT GCG GCG TAC AAG CTG GGA AGT GCG ATG AGC AGG CCG ATC ATA CAT TTC GCG AGT GAC TAT GAG CAG CCG TAC TAT CCG GAA AAC ATG CAC CCG TAC CCG
 Gly Leu Gly Gly Trp MET Leu Gly Ser Ala MET Ser Arg Pro Ile Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn MET His Arg Tyr Pro
 540 570 600
 AAC CAA GTG TAG TAC AGG CCG ATG GAT GAG TAC AGC AAC CAG AAC AAC TTT GTG CAG GAC TGC GTC AAT ATC ACA ATC AAG CAG CAC ACG GTC ACC ACA ACC ACC
 Asn Gln Val Tyr Tyr Arg Pro MET Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
 630 660 690 720
 AAG GCG CAG AAC TTC ACC GAG ACC GAC GTT AAG ATG ATG GAG CCG GTG GTT CAG CAG CAG ATG TGT ATC ACC CAG TAC GAG AGG GAA TCT CAG GCG TAT TAC CAG AGA
 Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys MET MET Glu Arg Val Val Glu Gln MET Cys Ile Thr Gln Tyr Glu Arg Ser Gln Ala Tyr Tyr Gln Arg
 750 780 810
 GGA TCG AGC ATG GTC CTC TTC TCC TCT CCA CCG GTC ACC CAC CCG ATG TCT TTT CTC ATC TTC CTC ATC CTG GGA TGA GGA AGG TCT TCC TTT CAC CAT CTT
 Gly Ser Ser MET Val Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
 840 870 900 930
 TCT AAT CTT TTT CCA GCT TGA GCG AGG CCG TAT CCA CCG GCA GCG CTT TTA GTC GTC GTC TCT CAG TCT TTC TTC TCT CTT TGT CCC GGA TAG GCT AAT CAA TAC
 960 990 1020
 CCT TGG CAG TGA TGG GCA CTG GAA AAC ATA GAG TAG AGC TGA GAT GCT GGT CAA GCG CCG TTT GAT TGA GTT CAT CAT GAG CCG TTG CTA ATG CCA GCG CAG TAA
 1050 1080 1110 1140
 AAG TAT AAC AGC AAA TAA CCA TGG GTT AAT CCG GAC TTA TTT TGG GAC TTA CCG GAA CAG GTT GAG CCG AAA ACA AAT CTC AGA ACA GTC TGA AAT ACC TTT GCG
 1170 1200 1230
 TGG ATA CCT CCG GCT CCG TCA GCA CCG AGA GCT CAG TAT ACT AAT GCG CTA TCT TAG TAG AGA TTT CAT AGC TAT TTA GAG ATA TTT TCC ATT TTA AGA AAA CCC
 1260 1290 1320 1350
 GAC AAC ATT TCT GCG AGG TTT GTT AGG AGC CCA CAT GAT ACT TAT TCA AAA AAA TCC TAG AGA TTC TTA GCT CTT GCG ATG CAG GCT CAG CCG GCT GGA GCA TGA
 1380 1410 1440
 GCT CTG TGT GTA CCG AGA ACT GGG GTG ATG TTT TAC TTT TCA CAG TAT GCG CTA CAC AGC AGC TGT TCA ACA AGA GTA AAT ATT GTC ACA ACA CTG AAC CTC TGG
 1470 1500 1530 1560
 CTA GAG GAC ATA TTC ACA GTG AAC ATA ACT GTA ACA TAT ATG AAA GCG TTC TGG GAC TTG AAA TCA AAT GTT TGG GAA TGG TGC CCT TGG AGG CAA CCT CCC ATT
 1590 1620 1650
 TTA GAT GTT TAA AGG ACC CTA TAT GCG GCA TTC CTT TCT TTA AAC TAT AGC TAA TTA AGC CAG CTC AAA AGT AAA TTC CCG TCT AGA CAC TGA AGG CAA ATC TCC
 1680 1710 1740 1770
 TTT GTC CAT TTA CCG GCG AAC CAG AAT GAT TTT GAC ATA CAG GAG AGC TCG ACT TGG GAA AGC ACC ATC ATC ATA GAG GAT GAT GTA ATT AAA AAA TGG TCA CTG
 1800 1830 1860
 TGC AAA GAA AAG AAC TGC TTG CAT TTC TTT ATT TCT GTC TGA TAA TTT TCA AAA ACC AGA AAT AGC TCA AGT TCA TAG TTT CTG TAA TTG GCT TTT GAA TCA AAG
 1890 1920 1950 1980
 AAT AGG GAG ACA ATC TAA AAA ATA TCT TAG GTT GGA GAT CAC ACA AAT ATG ATT GAT TTG AAG TGG AAA AAG AAA TTC TCT TAA TGT TAA TTA AAG TAA AAT TAT
 2010 2040 2070
 TCC CTG AAT TCT TTG ATA TTG TCA CCG AGC AGA TAT GTA TTA CTT TTC TGC AAT GTT ATT ATT GCG TTG CAC TTT GTC AGT ATC TAT GTA AAA ATA TAT ATG TAT
 2100 2130 2160 2190
 ATA AAA TAT ATA TTG CAT AGG ACA GAC TTA GGA GTT TTG TTT ACA GCA CTT AAC ATG TGA AGT GTC TAT TGC ATT AAC TTT TGT AAG GTA CTG AAT ACT TAA TAT
 2220 2250 2280
 GTG GGA AAC CCG TTT GCG TGG TCC TTA GCG TTT CAG TGT GCA CAG CTT GGT TTC ATC TAA CAA TCC AAA GIG GAC ACC ATT AAC AGG TCT TTG AAA TAT GCA TGT
 2310 2340 2370 2400
 ACT TTA TAT TTT CTA TAT TTG TAA TTT TGG ATG TTC TTG TTT TGT TAT ATA AAA AAA TAG TAA ATG TTT AAT ATC TGA CTG AAA TTA AAC GAG CGA AGA TGA GCA
 2432
 CCA AAA AAA AAA AAA AAA A

FIG. 3. Nucleotide sequence of the human PrP cDNA (consensus sequence) and the deduced amino acid sequence. Due to a compression, T in position 1965 is ambiguous.

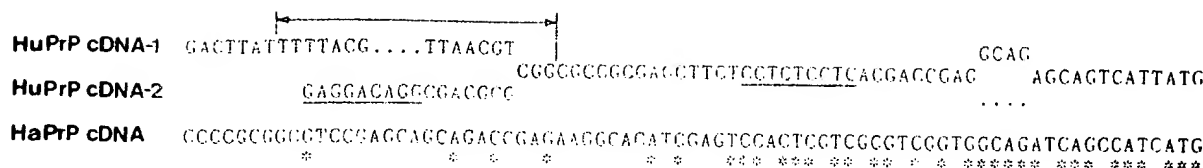


FIG. 4. Consensus sequence of HuPrPcDNA. A consensus sequence was established by exclusion of incongruent sequences at the 5' ends of HuPrPcDNA-1 and HuPrPcDNA-2. Nucleotides in bold letters represent the ends of a presumed cloning artefact. This presumptive artefact corresponds to the reverse complement of nucleotides 651–1050 in Fig. 3. Underlined nucleotides in HuPrPcDNA-2 and the consensus sequence might represent a similar artefact. A comparison of HuPrPcDNA-2 and the consensus sequence with the 5' untranslated region of the hamster shows that 24 of 33 nucleotides immediately preceding the first ATG codon (shown at the right margin) are identical. These are indicated by asterisks. The entire ORF of HuPrPcDNA-2 has been sequenced and was found to be identical with the ORF of HuPrPcDNA-1. In the 5' untranslated region, the nucleotides GCAG are absent in HuPrPcDNA-2 and are represented by dots.

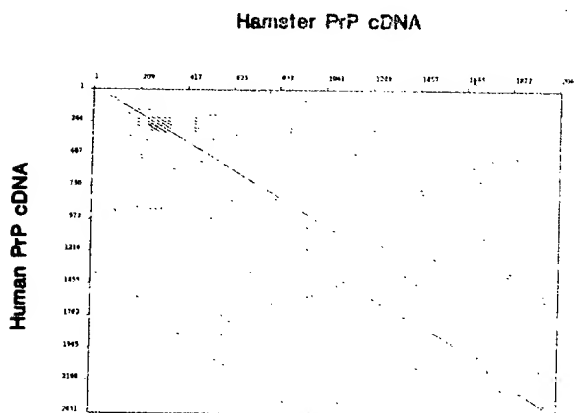


FIG. 5. Comparison of the nucleotide sequences of the human and hamster (Oesch *et al.*, 1985; K. Basler, B. Oesch, M. Scott, D. Westaway, M. Wälchli, D.F. Groth, M.P. McKinley, S.B. Prusiner, and C. Weissmann, submitted) PrP cDNA sequences. Stretches of eight identical nucleotides in both sequences appear as short bars.

matches in the second codon position, and 70 are mismatches in the third codon position. Fifty-eight of these mismatches are silent changes. Seven of the amino acid differences occur among the 22 residues of the putative signal sequence (Fig. 5), which is not surprising, since signal peptide sequence variation is well documented (Sabatini *et al.*, 1982; von Heijne, 1983). When the signal peptide is excluded, the hamster and human PrP proteins exhibit 91.34% (211 out of 231) amino acid sequence homology, with most of this divergence occurring within the carboxy-terminal half of the molecule. Eighteen of the 27 amino acid substitutions are conservative; only nine amino acid substitutions are nonconservative (Table 1). The degree of sequence conservation between hamster and human PrP is consistent with filter hybridization experiments which reveal that rat, sheep, goat, nematode, *Drosophila*, and possibly yeast harbor candidate PrP gene sequences (Westaway and Prusiner, 1986). Conservation extends to the chromosomal location of the prion protein gene which has been assigned to chromosome 20 in humans and the corre-

sponding homologous chromosome 2 in mice (Sparks *et al.*, 1986). This broad spectrum of phylogenetic conservation suggests that PrP molecules have an important function in cellular metabolism.

The amino-terminal portion of the human prion protein contains two short repeats of GG(N/S)RYP beginning at amino acids 34 and 45 (Fig. 6). These are followed by five long repeats of P(H/Q)GGG(-/G)WGQ beginning at amino acid 51. Conservation of these repetitive motifs between the human and hamster PrP gene is perfect except for one substitution at residue 56 (Fig. 6). The high degree of conservation of these repeats suggests that they are important for the function of the cellular PrP isoform. However, they do not appear to be required for the transmission of scrapie since they are lost when the hamster scrapie prion protein isoform (PrP 33-35S) is digested *in vitro* with proteinase K generating PrP 27-30. Proteinase K digests the hamster scrapie prion protein between residues 130 and 131, since the amino terminus of PrP 27-30 is the glycine at residue 131 (Prusiner *et al.*, 1984; Oesch *et al.*, 1985) (Fig. 6).

The predicted structure of the human and hamster prion proteins is consistent with their suspected role in the pathogenesis of prion diseases. In these diseases, the nerve cell membrane appears to be the primary target since the characteristic ultrastructural feature is dilatation of neurites in the grey matter in association with focal necrosis and splitting of the neuronal cell membrane (Chou *et al.*, 1980). The predicted amino acid sequence suggests that prion proteins are membrane-bound since they contain two highly hydrophobic regions capable of spanning the membrane (Oesch *et al.*, 1985). The first, from amino acid 112 to 134, is long enough to span the unit membrane in a helical configuration and has two positively charged residues at its amino end which could anchor it to the membrane surface. The second is at the carboxy terminus of the protein and has the potential to anchor this end of the protein in the membrane. Studies of the transmembrane orientation of the prion proteins are in progress (B. Hay, R. Barry, I. Lieberburg, S.B. Prusiner, and V. Lingappa, in preparation; J.F. Bazán, R.J. Fletterick, M.P. McKinley, and S.B. Prusiner, in preparation). Hypothetically, the accumulation of abnormal amount of the proteinase-resistant prion protein

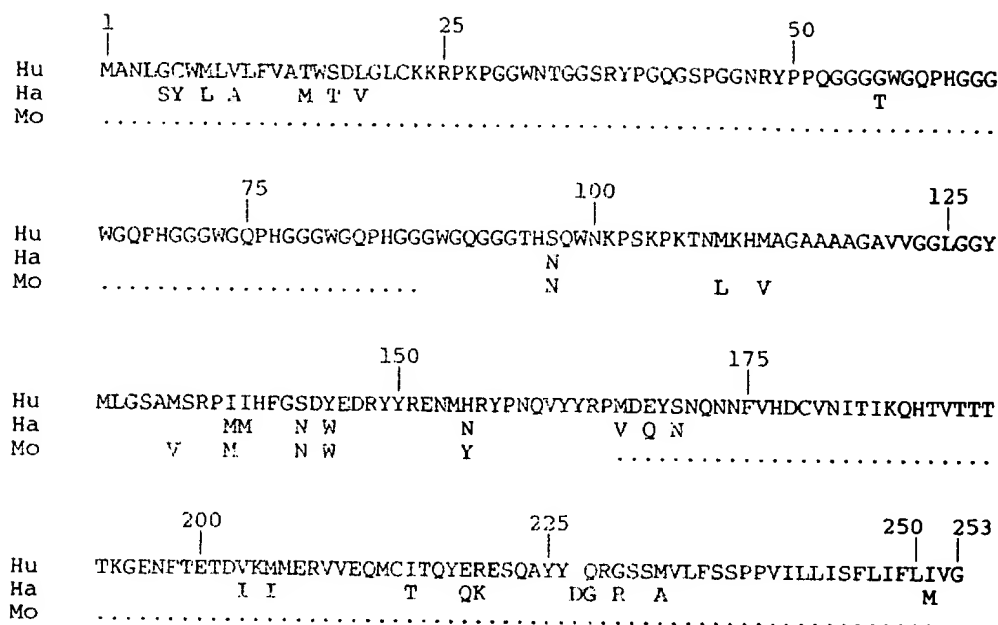


FIG. 6. Comparison of the human PrP sequence (Hu) with the hamster (Ha) (Oesch *et al.*, 1985; Basler *et al.*, submitted) and the mouse equivalents (Mo) (Chesebro *et al.*, 1985). Identical amino acids are not shown. Only amino acid replacements are presented. Unknown parts of the mouse sequence are represented by dots.

in neuronal cell membranes might lead to spongiform degeneration of neurons. Indeed, immunohistochemical studies in the hamster have shown increased concentrations of the scrapie prion protein specifically in grey matter areas undergoing spongiform degeneration (S.J. DeArmond, D. DeMott, R.A. Barry, H.A. Kretschmar, and S.B. Prusiner, in preparation).

The propensity of the scrapie prion protein to polymerize into filaments which form amyloid plaques during scrapie (DeArmond *et al.*, 1985) and of antigenically related proteins to form into amyloid plaques in CJD and GSS, has been established by peroxidase immunohistochemistry with anti-PrP 27-30 antiserum (Kitamoto *et al.*, 1986). In addition, purified PrP 27-30 forms into rod-like structures *in vitro* which are morphologically similar to the structures formed by proteins purified from other amyloids (Prusiner *et al.*, 1983). The prion protein rods also have the histochemical characteristics of amyloid, that is, they bind Congo Red dye which displays green birefringence in polarized light (Prusiner *et al.*, 1983). It is of interest that the hamster and human prion proteins have potential amphipathic helical regions similar to amyloid protein A that occurs in systemic amyloidosis secondary to chronic diseases such as tuberculosis. The amphipathic region of amyloid A protein can assume an α -helical configuration with an apolar face and a polar face, the latter containing charged amino acid residues (Segrest *et al.*, 1976; Lücke, 1981). The prion proteins contain two potentially amphipathic regions between the two hydrophobic transmembrane segments (J.F. Bazán, R.J. Fletcher, M.P. McKinley, and S.B.

Prusiner, in preparation). Amphipathic sequences are also characteristic of apolipoproteins (Segrest *et al.*, 1974) which, by analogy, suggests that the prion protein could bind to the polar ends of membrane phospholipids.

Knowledge of the human PrP cDNA sequence will allow construction of synthetic peptides in regions apparently unique to man. Monospecific antisera raised against these peptides should distinguish human from rodent PrP molecules. Such reagents will be useful in testing models of prion replication. For example, mice are experimental hosts for both scrapie and CJD prions. It will be interesting to examine whether prions obtained after passage through mice exhibit human- or rodent-specific PrP determinants. In addition, human monospecific antisera may offer advantages over the present heterologous antisera for analysis and diagnosis of human transmissible encephalopathies.

Considerable evidence is accumulating which shows that the scrapie, CJD, and GSS prion proteins are abnormal isoforms of neuronal membrane proteins encoded by a cellular gene and that the formation of those proteins is a central pathogenic event in prion diseases. This hypothesis is consistent with current knowledge about these diseases including observations showing that 10% of CJD cases are familial and GSS cases are dominantly inherited. These diseases, therefore, appear to be primary degenerative disorders of neurons caused by the accumulation of the abnormal prion protein isoform. The unique feature of these diseases is that the prion protein is also a major constituent of prions.

TABLE 1. NUCLEOTIDE MISMATCHES AND PREDICTED AMINO ACID REPLACEMENTS BETWEEN HUMAN AND HAMSTER cDNAs

<i>Mismatch codon position</i>	<i>Amino acid position</i>	<i>Amino acid human-hamster</i>	<i>Polarity human-hamster^a</i>
1	5	G-S	O - O
	8	M-L	⊙ - ⊙
	155	H-N	⊕ - O
	166	M-V	⊙ - ⊙
	168	E-Q	⊖ - O
	219	E-Q	⊖ - O
1 + 2	232	M-A	⊙ - ⊙
1 + 3	19	L-V	⊙ - ⊙
	203	V-T	⊙ - O
	229	G-R	O - ⊕
1 + 2 + 3	56	G-T	O - O
	*	D	⊖
	227	Q-G	O - O
2	6	C-Y	O - C
	17	S-T	O - O
	97	S-N	O - O
	143	S-N	O - O
	170	S-N	O - O
	215	I-T	⊙ - O
	220	R-K	⊕ - ⊕
2 + 3	10	V-A	⊙ - ⊙
	15	T-M	O - ⊙
	145	Y-W	O - ⊙
3	138	I-M	⊙ - ⊙
	139	I-M	⊙ - ⊙
	205	M-I	⊙ - ⊙
	251	I-M	⊙ - ⊙

^a ⊕, Positively charged; ⊖, negatively charged; O, polar, uncharged; ⊙, hydrophobic; *, no equivalent in human cDNA.

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